Transcript of ASO Hybridisation Video
https://www.youtube.com/watch?v=laAuplFhE7U&t=147s

ASO hybridisation It is a common tool in molecular biology, genetic testing or forensic research.

In the human disease sickle cell, there is a genetic mutation in the blood protein beta-haemoglobin. ASO can be used to detect the mutation in a DNA sample and therefore is used to identify the presence of the sickle cell mutation.

ASO hybridisation

ASO hybridisation depends on the fact that complimentary stretches of DNA can form stable hybrids (or double stranded pieces of DNA). We see this for the Beta A allele (which would be present in healthy haemlobin) and the beta S allele which would feature in sickle cell. The two sequences that are 100% complimentary will form two stable hybrids.

Shown are portions of two alleles of the b–globin gene in double stranded form. (the usual b^A allele and the mutated b^A allele) Codon 6, or the 6th amino acid, is where the sickle cell mutation occurs, changing from gAg to gTg as highlighted in red. So you can see the gAg codon on the Beta A allele compared to the GTG codon of the Beta S allele.

Above and below the two alleles are two “probes” that we can form in the lab; these are single-stranded pieces of DNA that are complimentary to one strand of each allele. These are the allele-specific oligonucleotides (ASO), which are labelled (as shown by the *) so that they can subsequently be detected by a suitable laboratory technique.

Regions of DNA that contain codon 6 (the target DNA) are then specifically amplified using polymerase chain reaction (PCR). The resulting amplified DNA is made single stranded and “fixed” to a solid support (a piece of nylon) in dots in a technique called a Dot Blot. This can be done by simply pipetting the DNA on to the solid support and denaturing it to make it single stranded and fixing it on the membrane to prevent it being lost.

Diagrams diagrams A and B show experiments to hybridise ASOs to the target DNA. There are 3 samples present, the first with the normal allele ($b^A b^A$), the

second with the sickle allele (b^S b^S) and the third would be a carrier of sickle cell (b^A b^S).

The probe ASOs can now be hybridised to the target DNA on the identical solid supports. If a perfectly complimentary sequence is present on the solid support a stable hybrid molecule will be formed which can be detected by virtue of the label included on the ASO. In this example the presence of a stable hybrid is indicated by a blue-colour. Oligonucleotides designed for ASO-hybridisation are short enough to ensure that with carefully controlled hybridisation conditions a single mismatch prevents the formation of a stable hybrid. Any unstable hybrids are washed off in the procedure and no colour remains.
In the diagram, the results show that the b^A Probe (left) has formed a stable match with alleles 1 and 3 and shows up blue. The b^S probe (right) matches with with 2 and 3.

So ASO analysis is one laboratory method used to detect genetic mutations.

- Wikipedia 'Allele specific oligonucleotide' article. Available: http://en.wikipedia.org/wiki/Allele-specific_oligonucleotide
- Wu et al (1989) Allele-specific enzymatic amplification of beta-globin genomic DNA for diagnosis of sickle cell anemia. Available: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC286997/